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A Microarray Immunoassay for Simultaneous Detection of Proteins and Bacteria

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We report the development and characterization of an antibody microarray biosensor for the rapid detection of both protein and bacterial analytes under flow conditions. Using a noncontact microarray printer, biotinylated capture antibodies were immobilized at discrete locations on the surface of an avidin-coated glass microscope slide. Preservation of capture antibody function during the deposition process was accomplished with the use of a low-salt buffer containing sucrose and bovine serum albumin. The slide was fitted with a six-channel flow module that conducted analyte-containing solutions over the array of capture antibody microspots. Detection of bound analyte was subsequently achieved using fluorescent tracer antibodies. The pattern of fluorescent complexes was interrogated using a scanning confocal microscope equipped with a 635-nm laser. This microarray system was employed to detect protein and bacterial analytes both individually and in samples containing mixtures of analytes. Assays were completed in 15 min, and detection of cholera toxin, staphylococcal enterotoxin B, ricin, and *Bacillus globigii* was demonstrated at levels as low as 8 ng/mL, 4 ng/mL, 10 ng/mL, and 6.2 × 10^4 cfu/mL, respectively. The assays presented here are very fast, as compared to previously published methods for measuring antibody−antigen interactions using microarrays (minutes versus hours).

The development of arrays of immobilized biological compounds in micrometer-sized spots on a surface (microarrays) has played an increasingly important role in modern biology and medicine.[1-3] Microarrays offer several distinct advantages over conventional analytical devices: they are small, manufacture can be automated, and use requires only small sample and reagent volumes. Perhaps the biggest advantage offered by microarrays is the possibility for massively parallel analysis. Although significant progress has been made in the production and application of DNA microarrays,[4-7] the sequencing of the human genome has placed an ever-increasing emphasis on elucidating the functions of the myriad proteins encoded by these genes. As a result, protein microarrays have emerged as a means to address this question.[8,9] To date, the majority of protein microarrays have utilized antibodies, which offer specificity and often high affinity, as the reagent of choice. Antibody microarrays have been used successfully to detect and quantify specific target proteins in complex mixtures,[10] to screen and identify differentially expressed proteins produced by recombinant antibody technology,[11] and to detect clinically relevant cytokines.[12,13] Many antibody microarray assays continue to take advantage of the proven utility of ELISA (enzyme-linked immunosorbent assay) in either the indirect format in which the antigen is immobilized[14] or in the sandwich format.[13] However, the ELISA technique is often subject to long incubation times and multiple wash steps that lengthen assay time and limit throughput.

Our laboratory previously reported the development of an array biosensor that utilizes sandwich immunoassays performed under flow conditions to detect protein, bacterial, and viral analytes rapidly.[15-18] In these studies, capture antibody spots (2.5 mm^2) were generated by physically isolated patterning using polymer flow cells.[16] In this report, we describe the development of an antibody microarray biosensor composed of capture antibody spots (0.041 mm^2) produced by printing with a piezoelectric noncontact microarrayer. Capture antibody function was preserved by depositing the capture molecules in a low-salt printing buffer containing sucrose and the carrier protein bovine serum albumin.

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This buffer effectively protected the antibodies against dehydration and denaturation. The flow-based antibody microarray biosensor offers the advantage of a rapid, 15-min assay, as compared to other antibody microarray assays that often take hours to complete. However, the rapid assay format is realized without sacrificing sensitivity. We demonstrate this by constructing assays for the protein analytes cholera toxin (CT), staphylococcal enterotoxin B (SEB), and ricin and the bacterium *Bacillus globigii*. Detection of each analyte is demonstrated at levels as low as those achieved by other biosensor technologies. In addition, whereas other antibody microarray assays have demonstrated the ability to detect specific proteins present in complex mixtures, [9] we believe this report to be the first instance in which an antibody microarray is used to achieve the simultaneous detection of proteins and bacteria.

EXPERIMENTAL SECTION

Antibodies and Analytes. Unless otherwise specified, all chemicals were of reagent grade and used as received. Rabbit anti-SEB polyclonal antibody (pAb) and SEB antigen were purchased from Toxin Technology (Sarasota, FL). Mouse anti-SEB monoclonal antibody (mAb, clone 6B) was obtained from IGEN International (Gaithersburg, MD). Goat and rabbit anti-CT pAbs were purchased from Biogenesis, Inc. (Brentwood, NH). Mouse anti-CT mAb (clone 3D11) was a product of Biodesign International (Saco, ME). CT antigen was obtained from Calbiochem (La Jolla, CA). Goat anti-ricin pAb was a generous gift of Mr. Woody Johnson (Naval Medical Research Center (NMRC), Bethesda, MD). Ricin antigen was purchased from Sigma Chemical Co. (St. Louis, MO). Rabbit anti-*Bacillus globigii* pAb and *B. globigii* antigen (whole cell preparation) were generously supplied by Mr. Tom O'Brien (NMRC). Bovine serum albumin (BSA) and polyoxyethylenesorbitan monolaurate (Tween-20) were obtained from Sigma (St. Louis, MO). All other chemicals were obtained as noted in the text.

Appropriate safety precautions were exercised when handling bacterial and toxin preparations. All solutions, glassware, etc., containing bacteria or toxic analytes were handled by personnel wearing gloves and appropriate protective gear (lab coat and goggles). All equipment, benchtops, and instruments were disinfected with a 20% bleach solution and were rinsed with distilled water. Analyte-containing solutions were also treated with 20% bleach prior to disposal. Contaminated disposables (test tubes, pipet tips, used microarray chips) were placed in biohazard containers and later incinerated.

Preparation of Capture and Detection Antibodies. All microarray assays were performed using a standard sandwich immunoassay format. Antibodies employed as capture reagents were biotinylated for immobilization to the NeutrAvidin-coated microarray surface. Briefly, antibody and biotin–LC–NHS ester (Pierce Chemical, Rockford, IL) were reacted at a 5:1 molar ratio for 1 h at room temperature. Biotinylated antibody was separated from unincorporated biotin by size-exclusion chromatography using BioGel P-10 (Bio-Rad, Hercules, CA). Antibodies used as detection reagents were labeled with Cy5 (bisfunctional derivative, Amersham Life Sciences, Arlington Heights, IL) by reacting the antibody and Cy5 at a 5:1 molar ratio for 30 min at room temperature. Unincorporated Cy5 dye was removed from labeled protein by size-exclusion chromatography. The Cy5/antibody ratios ranged from 2.8 to 4.5 for all antibody preparations.

Preparation of Antibody Microarrays. To prepare slides for microarrays, conventional glass microscope slides (Daiggerbrand, Wheeling, IL) were scrupulously cleaned by immersion in KOH (10% w/v) in methanol for 30 min, followed by copious rinsing with deionized water and drying under a nitrogen stream.[19] The cleaned slides were silanized under a nitrogen atmosphere by immersion for 1 h in 3-mercaptopropyl triethoxysilane (2% v/v) in anhydrous toluene. The slides were washed in anhydrous toluene and nitrogen-dried. The silanized slides were immersed in 1 mM *N*-(γ-maleimidobutyryloxy)succinimide ester (GMBS, Pierce), prepared by dissolving 12.5 mg of GMBS in 0.25 mL DMSO and then diluting in 43 mL of absolute ethanol, for 30 min at room temperature. The slides were washed with water and incubated in 1.5 μM NeutrAvidin Biotin-Binding Protein (Pierce) in phosphate buffer (10 mM sodium phosphate, 10 mM NaCl, pH 7.4) overnight at 4 °C. The NeutrAvidin-coated slides were rinsed in phosphate-buffered saline (PBS; 137 mM NaCl, 3 mM KCl, 10 mM phosphate, pH 7.4) and stored in blocking buffer (10 mM phosphate, 10 mM NaCl, 1% (w/v) BSA, 0.01% (w/v) NaN_3) at 4 °C. The slides could be stored in this manner for up to 4 weeks prior to use. Immediately prior to array printing, the slides were removed from blocking buffer and rinsed with PBS, followed by rinsing with deionized water. The rinsed slides were then dried under a stream of nitrogen.

Biotinylated capture antibodies were prepared in deposition buffer (10 mM phosphate, 10 mM NaCl, 10 mM sucrose, 0.1% (w/v) BSA) at a final concentration of 10 μg/mL and were deposited onto the NeutrAvidin-coated surface using a BioChip Arrayer I (Packard Bioscience, Meriden, CT) housed in an atmospherically isolated chamber with a relative humidity of 45% and a temperature of 23 °C. The arrayer was programmed to deliver antibody solutions at 1 nL/spot and consistently delivered spots ~230 μm in diameter. Capture antibodies were deposited in a horizontal orientation at discrete positions within each sample row on the surface of the slide (Figure 1A). Immediately after printing, the position of each row of spots was marked for later alignment with the multiple-channel flow chamber module (see below). The slides were then immersed in blocking buffer for 20 min at 25 °C. Alternatively, the slides could be stored in blocking buffer at 4 °C for up to one week prior to use.

Sample Analysis. Immediately prior to assay, the slides were removed from blocking buffer and were rinsed twice with PBS–Tween (PBST; PBS containing 0.05% (v/v) Tween-20). A multiple-channel poly(dimethylsiloxane) (PDMS; Liquid Silicone Elastomer, NuSil Technology, Carpenteria, CA) flow module was then aligned onto the slide such that each channel encompassed a row of deposited spots (Figure 1B). Each channel measured 22 × 1.5 mm and a 2-mm spacer separated each channel. The production of the PDMS flow modules is described in detail in Rowe et al.[16] All solution transfers through the flow module were achieved using a peristaltic pump. Each channel was rinsed by flowing 1 mL PBST-BSA (PBSTB; PBST containing 0.1% (w/v) BSA) through the channel at a flow rate of 1 mL/min. After rinsing, 0.3 mL of sample (in PBSTB) was flowed through the channel at a

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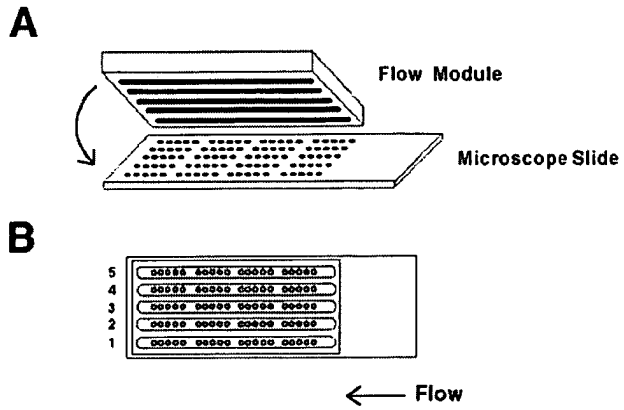

Figure 1. Antibody microarray printing and assay format. (A) Biotinylated capture antibodies are deposited at discrete locations within a sample row and are immobilized on the glass surface by noncovalent association between biotin and avidin. (B) A multichannel flow module is aligned on the deposited antibody array. Sample, detection antibodies, and wash buffers are sequentially introduced at one end of the chamber and are drawn over the microarray surface using a peristaltic pump. After washing, if an analyte is present in a sample, the appropriate spots fluoresce.

rate of 0.3 mL/min. The channel was rinsed again with PBSTB as described above. An aliquot (0.3 mL) of Cy5-labeled detection antibodies (final concentration of 2.5−10 μg/mL in PBSTB) was then flowed through the channel at a flow rate of 0.3 mL/min. After rinsing twice with PBSTB, the PDMS flow module was removed, and the slide was rinsed twice with PBST. Finally, the slide was rinsed with deionized water and dried under a stream of nitrogen prior to imaging. Assays were typically completed in 15 min.

Microarray Imaging and Data Analysis. All microarrays were imaged using a ScanArray Lite microarray scanner (Packard BioChip Technologies, Billerica, MA) equipped with a 635-nm laser with 10 μm pixel resolution. The fluorescence intensity of the two-dimensional array of spots was determined using the QuantArray microarray analysis package (version 2.1, Packard BioChip Technologies). For all analyses, the fixed circle quantitation method resident within the software was used to locate and quantify the fluorescence intensity of each spot and its surrounding background. Each spot in the array was encompassed by a circular spot mask (230 μm in diameter) and a background mask (inner diameter of 240 μm, outer diameter of 300 μm). Spot intensity was determined as the mean intensity of the pixels within the spot mask minus the mean intensity of the pixels located within the background mask.

RESULTS AND DISCUSSION

Microarray Printing and Performance. The production of protein microarrays of high quality necessitates the careful preparation of both the protein sample and the printing surface.[20] In this study, several procedures were implemented to preserve the activity of the capture antibodies during array fabrication. To protect against the potential denaturing effects of a low protein concentration environment, the composition of the deposition

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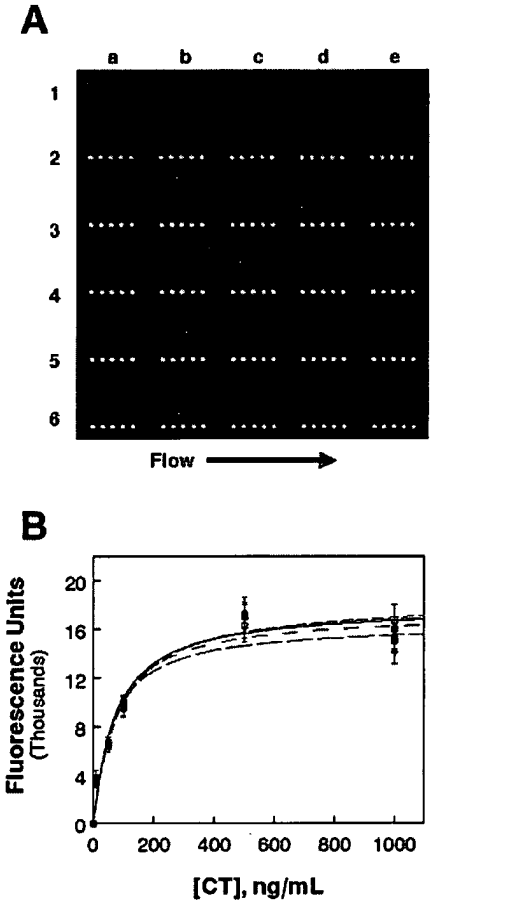

Figure 2. Representative microarray for the detection of a single analyte. (A) Detection of cholera toxin (CT). CT was flowed through separate sample channels at concentrations of 0, 8, 50, 100, 500, and 1000 ng/mL (rows 1–6, respectively) over spots of immobilized biotin goat anti-CT antibody. Each sample row contained five subarrays (columns a–e) consisting of five capture antibody spots each. After exposure of the array to Cy5-rabbit anti-CT antibody, the array was washed and imaged using a microarray scanner. (B) Dose–response curves for CT. Fluorescence values were plotted for each subarray at each CT concentration. Each data point represents the mean ± SD for the five spots within each subarray. Symbols for each subarray are: a (+), b (□), c (○), d (■), e (●). For each data set, a single-site ligand binding function (included in Slidewrite for Windows version 5.01 (Advanced Graphics Software, Encinitas, CA)) was used to draw the best line through the data points.

buffer was amended to a final protein concentration of 1 mg/mL by the addition of carrier protein (bovine serum albumin). Additionally, sucrose was included in the deposition buffer to protect the antibodies against the denaturing effect of dehydration during the printing process. Other investigators have added glycerol to their deposition buffers to prevent evaporation of the nanoliter droplets.[3] It has been our experience that the omission of carrier protein or a dehydration protectant during array fabrication yields antibody arrays of poor quality and reproducibility (Delehanty, unpublished results).

Detection of Individual Protein and Bacterial Analytes. To investigate the performance of the antibody microarray format, it was of interest to first determine the array response for the detection of individual analytes. Figure 2A shows a representative response obtained when a sandwich immunoassay was performed

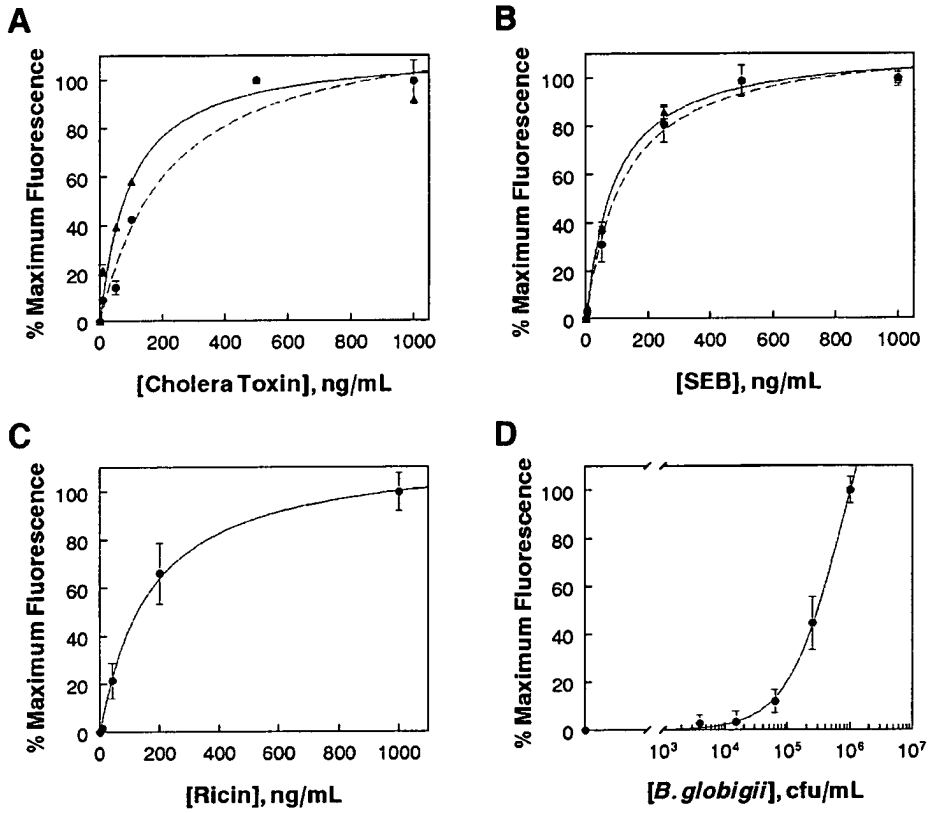

Figure 3. Flow antibody microarray response for the detection of single analytes. Microarray assays were performed as described in the Experimental Section for each analyte at the indicated concentrations. (A) CT detected with (▲) Cy5-rabbit anti-CT or (●) Cy5-mAb 3D11; (B) SEB detected with (▲) Cy5-rabbit anti-SEB or (●) Cy5-mAb 6B; (C) Ricin detected with (●) Cy5-goat anti-ricin; (D) *B. globigii* detected with (●) Cy5-rabbit anti-BG (note log scale). Each data point represents the mean ± SD of all five subarrays at each concentration tested. Results are plotted as percent maximum fluorescence. Capture and detection antibody pairs, calculated limits of detection (LOD), and the lowest concentrations for which detection was demonstrated are summarized in Table 1. Concentrations of each detection antibody used are noted in the text. The curves were fit through the data points as described in the legend for Figure 2B.

on the microarray for the detection of a single analyte. The image shows the resulting fluorescence pattern that was generated when CT was flowed over five sequential subarrays of immobilized biotinylated goat anti-CT antibody (columns a–e) at concentrations of 0, 8, 50, 100, 500, and 1000 ng/mL (rows 1–6, respectively). Detection of the bound analyte was subsequently achieved using Cy5-rabbit anti-CT pAb at a final concentration of 2.5 μg/mL. It was apparent that an increase in CT concentration resulted in a corresponding increase in the fluorescence emitted from the arrayed spots. As the concentration of CT increased, the color intensity changed from low-intensity false-colored blue (row 2; 8 ng/mL CT) to high-intensity false-colored green (row 6; 1000 ng/mL CT). The fluorescence resulting from the buffer blank row (row 1; 0 ng/mL CT) was minimal (<6% of the fluorescence generated by the lowest concentration tested), indicating a small degree of nonspecific binding of the Cy5-rabbit anti-CT antibody to the immobilized capture antibody in the absence of analyte. The average fluorescence resulting from spots within the buffer blank row was subtracted from all values present in the array to yield the blank-corrected fluorescence values that were subsequently plotted to generate dose–response curves. The dose–response curves in Figure 2B were generated by plotting the mean fluorescence intensity ± standard deviation (SD) for all five subarrays at each CT concentration tested. The calculated limit of detection (that concentration corresponding to three SD above the background) was determined to be 0.3 ng/mL. The lowest concentration for which detection was demonstrated was 8 ng/mL. Rowe Taitt et al.[18] reported detection of CT at levels as low as 1.6 ng/mL using an array biosensor that employed evanescent wave fluorescence. In that study, the area of each patterned capture antibody spot was ~50 times the area for the spots described in the present investigation. The dynamic range of the microarray assay (the concentration range that gave the best fit to the linear equation $y = mx + b$) was from 8 ng/mL to 100 ng/mL ($R^2 = 0.970$). At concentrations above 100 ng/mL, the dose–response curve began to plateau. The intraslide variation in fluorescence (determined as the coefficient of variation (CV) among the five subarrays at each concentration) ranged from 3.4% at 100 ng/mL CT to 10.8% at 8 ng/mL CT. Assays performed on three independent slides demonstrated an average variation of 10.0% in the fluorescence values at each concentration tested (data not shown). However, the dose–response curves and the limits of detection were similar to those described above.

Similar sandwich microarray assays were developed for SEB, ricin, and *B. globigii*. Figure 3 shows the results of the dose–response curve analyses performed in this study. For each analyte, the capture and detection antibody pairs, the calculated limits of detection, and the lowest concentration for which detection was

Table 1. Summary of Single Analyte Detection of Protein and Bacterial Analytes by Antibody Microarray

antigen	biotinylated capture antibody	Cy-5-labeled detection antibody	calcd limit of detection (LOD)[a]	lowest concn detected above LOD
CT				
	goat pAb IgG	rabbit pAb IgG	0.3[b]	8.0[b]
	goat pAb IgG	mAb 3D11	3.8[b]	8.0[b]
SEB				
	rabbit pAb IgG	rabbit pAb IgG	2.8[b]	4.0[b]
	rabbit pAb IgG	mAb 6B	3.0[b]	4.0[b]
ricin				
	rabbit pAb IgG	rabbit pAb IgG	9.1[b]	10[b]
B. globigii				
	rabbit pAb IgG	rabbit pAb IgG	6.2×10^4[c]	6.2×10^4[c]

[a] LOD is defined as 3 SD above background. [b] Units are ng/mL. [c] Units are cfu/mL.

demonstrated are summarized in Table 1. All of the curves are plotted as a percentage of the maximum fluorescence achieved in each assay. Where suitable reagents were available, it was of interest to compare the microarray response for a particular analyte when detected with different Cy5-labeled antibodies. Figure 3A (triangles) shows a replot of the data shown in Figure 2B in which CT was detected with Cy5-rabbit anti-CT pAb (described above). The data are shown plotted through 500 ng/mL CT, since the assay reached saturation at higher concentrations. Figure 3A (circles) shows the microarray response for the detection of CT when Cy5-labeled anti-CT mAb 3D11, at a final concentration of 10 μg/mL, was substituted for the Cy5-rabbit anti-CT pAb as the tracer antibody. The dynamic range of the assay was unchanged, and the CVs were similar to those obtained when the Cy5-rabbit pAb was used as the detecting reagent. However, the calculated limit of detection increased 12.5-fold to 3.8 ng/mL CT. Although mAb 3D11 was used at a 4-fold higher concentration relative to the rabbit pAb, it consistently yielded fluorescent signals that were 5-fold below those produced by the rabbit pAb (raw data not shown). The number of Cy5 fluorophores per antibody molecule was ~3.0 for both antibodies. Thus, the differences in fluorescence yield between the two antibodies cannot be explained by differences in the number of Cy5 molecules per antibody. Because both antibodies are directed against the β subunit of CT, it is likely that the decrease in sensitivity is attributable to the fact that mAb 3D11 recognizes only a single epitope, whereas the pAb recognizes multiple epitopes on the β subunit.

Figure 3B shows the dose–response curves obtained when SEB was detected with 5 μg/mL Cy5-rabbit anti-SEB pAb (triangles) or 10 μg/mL Cy5-anti-SEB-mAb 6B (circles). The microarray response was essentially the same regardless of the antibody used to achieve detection. At each SEB concentration tested, there was less than a 10% difference in the raw fluorescence values obtained when the two detection antibodies were compared (raw data not shown). Nonspecific binding of the fluorescent antibody to the immobilized capture antibody was not observed for either tracer antibody. As shown in Table 1, the calculated limit of detection with the rabbit pAb was 2.8 ng/mL SEB, but it was 3.0 ng/mL SEB with mAb 6B. For both tracer antibodies, the lowest concentration of SEB for which detection was demonstrated was 4 ng/mL. Rowe Taitt et al.[18] reported detection of 4 ng/mL for SEB in a 14-min assay using the evanescent wave array biosensor, and Anderson et al.[21] reported detection of 10 ng/mL using a fiber-optic biosensor.

The dose–response curve in Figure 3C shows the microarray response for ricin when it was detected with Cy5-goat anti-ricin pAb at a concentration of 2.5 μg/mL. The intraslide variation among the subarrays was lowest at 8 ng/mL ricin (CV equals 8.0%) and increased to 34.5% at 40 ng/mL and 19.2% at 200 ng/mL. The calculated limit of detection was determined to be 9.1 ng/mL. The lowest concentration for which detection was demonstrated was 10 ng/mL. This is comparable to the limit of detection reported in other biosensors for ricin.[18,21] The assay response was linear through 200 ng/mL ricin (R^2 = 0.991).

In Figure 3D is shown the dose–response curve for *B. globigii*. Bound analyte was detected using Cy5-rabbit anti-*B. globigii* antibody (5 μg/mL). The calculated limit of detection was determined to be 6.2×10^4 cfu/mL. The lowest concentration for which detection was demonstrated was also 6.2×10^4 cfu/mL. The dynamic range was from 6.2×10^4 cfu/mL to 1×10^6 cfu/mL, the highest concentration tested ($R^2 = 0.989$). This compares well with the results of Anderson et al.[21] who reported detection of *B. globigii* at levels of 5×10^4 cfu/mL. While other reports have appeared describing the use of microarrays of peptides for the detection of whole cells,[22] the detection of *B. globigii* in the flow antibody microarray format described here is notable. To our knowledge, it is the first report to describe of the use of a microarray to detect whole cells under flow conditions.

Simultaneous Detection of Multiple Analytes. Upon demonstrating that the flow antibody microarray could quantitatively detect individual protein and bacterial analytes, it next became of interest to characterize the ability to detect multiple analytes in the same sample. On a single microarray, five samples were assayed for the presence of four potential target analytes. Within each sample channel, capture antibody spots (8 replicate spots/analyte) were immobilized as described previously. Figure 4A shows the fluorescence pattern that resulted when five different samples containing either a single analyte (rows 1–4) or a mixture of all four analytes (row 5) were flowed over the arrayed capture antibody spots. Detection was achieved by exposing each sample channel to the following cocktail of Cy5-labeled antibodies directed against each of the target analytes: anti-CT mAb 3D11, anti-SEB mAb 6B, goat anti-ricin pAb, and rabbit anti-*B.globigii* pAb. The

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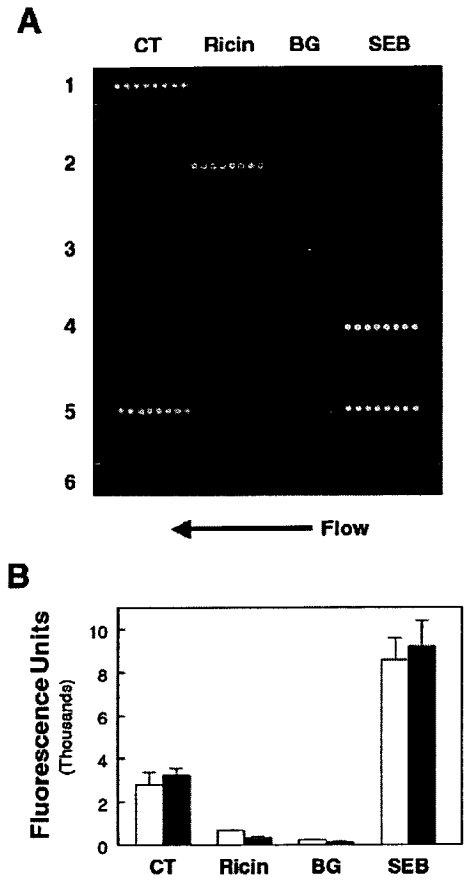

Figure 4. Flow antibody microarray response for the simultaneous detection of multiple analytes. (A) Each row of the microarray was patterned with eight replicate capture antibody spots directed toward four different analytes (noted above image). Four samples containing a single analyte (rows 1–4) and one sample containing a mixture of all four analytes (row 5) were flowed through each channel. In each row, bound analyte was detected with a cocktail of Cy5-labeled antibodies directed against the target analytes (described in the text). Concentrations of analytes: CT (row 1), 150 ng/mL; ricin (row 2), 250 ng/mL; *B. globigii* (row 3), 5×10^5 cfu/mL; and SEB (row 4), 250 ng/mL. Row 6 corresponds to zero analyte. (B) For each analyte, a comparison of the fluorescence signals in 4A, corresponding to either single analyte detection (open bar) or multiple analyte detection (solid bar), is shown. Data represent the mean ± SD of the eight capture antibody spots. (BG = *B. globigii*).

concentration of each antibody was 5 μg/mL except for the anti-ricin antibody, which was used at 2.5 μg/mL.

Figure 4A (rows 1–4) shows that each analyte produced distinct fluorescence signals at the appropriate site where its cognate capture antibody was immobilized. Row 5 shows the pattern of fluorescence that resulted when a mixture of all four analytes flowed through the channel.

A comparison of rows 1–4 reveals that the spots corresponding to *B. globigii* yielded the lowest fluorescence values and were ~30% as intense as the next-brightest spots (those corresponding to ricin). The most intense fluorescence was seen at the spots corresponding to SEB. This same trend was also seen in row 5, in which all four analytes were detected simultaneously. The extent of nonspecific binding was minimal. The graph in Figure 4B shows a comparison of the fluorescence signals derived when each analyte was detected either individually (open bar) or as part of a multianalyte assay (solid bar). The only analyte that exhibited a significant change was ricin. When detected as part of a multianalyte assay, the signals were 2-fold less than those obtained when ricin was detected individually. One possible explanation for this behavior is that when ricin and *B. globigii* are present in the same sample, ricin may bind to the peptidoglycan comprising the cell wall of the Gram-positive bacterium as a result of its capacity to function as a lectin, thus decreasing the effective concentration of ricin available to bind to the immobilized capture antibody.

CONCLUSIONS

Microprinting using inkjet and noncontact arrayers has the capacity to produce high-density arrays of proteins in very small spots. The purpose of this study was to use arrayer technology to make antibody arrays for the rapid and simultaneous analysis of multiple samples for multiple analytes. Protein and bacterial analytes were detected both individually and simultaneously using a rapid sandwich immunoassay that took 15 min to complete. Assays were constructed, and dose–response curves were generated for the protein analytes cholera toxin, staphylococcal enterotoxin B, and ricin, and the bacterium *B. globigii*. For all analytes, the lowest concentrations for which detection was demonstrated were comparable to those reported for other biosensor technologies. Detection of cholera toxin was demonstrated as low as 8 ng/mL. SEB was detected at concentrations as low as 4 ng/mL. For ricin, the lowest detected concentration was 10 ng/mL. The lowest concentration for which detection of *B. globigii* was demonstrated was 6.2×10^4 cfu/mL. We have also demonstrated that the microarray assay can detect the presence of multiple analytes in multiple samples with minimal nonspecific binding.

This work details several novel points related to antibody microarray assays. First, to our best understanding, this is the first study to describe the use of a microarray platform to achieve the simultaneous detection of both proteins and bacterial analytes under flow conditions. Second, the rapid nature of the assay system offers considerable advantages over more conventional antibody microarrays that require long incubation times. Finally, the automated deposition of antibodies in a buffer containing sucrose and carrier protein protects the capture molecules against dehydration and denaturation. This report has broad implications for the future development of antibody microarrays and for protein arrays in general. The model flow system described herein is amenable to high throughput, because only the number of sample flow channels that are introduced onto the array limits the number of samples that can be processed simultaneously. Furthermore, the number of potential analytes that can be queried is limited only by the geometric arrangement of the capture antibody spots within the sample channel.[23] In the current flow module design, each 22 × 1.5 mm sample channel could accommodate up to 88 capture antibody spots if printed as two rows with 0.5 mm spacing. Finally, the arrayer technology is much more amenable to commercial production of large numbers of "sensing chips" than previous methods for fabricating detector arrays.

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